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# CHANGES IN SOME MINERAL CONSTITUENTS OF PECAN NUTS AND THEIR SUPPORTING SHOOTS DURING DEVELOPMENT[1]

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## INTRODUCTION

The poor filling of pecan nuts has been recognized by various investigators (*2, 3, 4, 8, 11, 12, 14, 15, 16, 17, 19, 22*).[3] Different factors such as fertilizers, soil moisture, soil type, leaf area, and crop size have been suggested as having an influence on the filling of nuts, but in many respects the reports are contradictory.

Finch (*5*), studying pecan filling and maturity, found nut filling, date of maturity, and preharvest germination to be related to the vegetativeness, or greenness, of the tree during September and October. Vegetativeness seemed to be related to the available nitrogen supply. Later (*6*) he determined the nitrogen and phosphorus content of leaves, shucks, and nuts on four dates in the late stages of nut development and found an inverse relation between nitrogen and moisture in the plant and nut filling. There was no indication that poor filling was associated with a deficiency of phosphorus.

In more recent studies Finch and Van Horn (*7*) determined at intervals during the growing season the nitrogen content of pecan leaves from trees in various degrees of vegetativeness. They found a correlation between the nitrogen content of the leaves during the growing season and the quality of the nuts at harvest, but they concluded that nitrogen alone was not the direct controlling factor in nut filling. A closer correlation was found between the starch stored in fruit-bearing shoots preceding nut filling and the quality of the nuts produced. It was recognized that a number of factors other than nitrogen exerted an influence on the starch-storing ability of the tree and that cultural treatments would have to be considered in relation to environmental factors in order to achieve any considerable practical control over the filling of the nuts.

The writers (*10*) found differences in the filling of pecan nuts when fertilizers were applied at different times during the growing season. This indicated that the time of the intake of the minerals exerted an influence on the physiological processes of the tree. Therefore, the changes in the mineral constituents of pecan nuts during the course of their development and in the supporting shoots were studied in the hope of obtaining additional data on the cause of poor filling of nuts. The data obtained are reported herein.

[1] Received for publication March 10, 1943.

[2] Now with the Division of Rubber Plant Investigations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 305.

## MATERIALS AND METHODS

Samples were taken from 78 trees of the Moore variety of pecan, grown on Greenville sandy loam at Albany, Ga. Cultivation and green-manure crops were the same for all the trees; 68 of them were divided into 7 blocks and each block was fertilized at a different time during the growing season, while 1 block of 10 trees was not fertilized. A fertilizer containing 6 percent nitrogen, 8 percent phosphoric acid, and 4 percent potash was applied at the rate of 60 pounds per tree during 1935, 1936, and 1937. The nitrogen was reduced to 4 percent and the rate of application to 50 pounds per tree in 1938 and 1939. Dolomitic limestone was used in the fertilizer to make it nonacid-forming throughout the period.

The rainfall for the growing season in which the samples were taken was slightly above normal and well distributed. There was no evidence that the trees needed additional moisture at any time during the growing season. The trees appeared to be functioning normally, and the crop matured would seem to bear this out, as the trees produced an average of 76 pounds of nuts per tree. These nuts were of good quality, and 98 percent of them were of marketable grade. The mean oil content of the kernels was 73.1 percent.

On the four sampling dates the nuts were in the following stages of development:

July 15, 1939.—Nuts one-third to one-half full size. Shuck, shell, and kernel not yet distinguishable.

August 15, 1939.—Nuts about full size; shells hard; seed coat of kernel developed and filled with endosperm, but little or no discernible embryo formation.

September 15, 1939.—Shucks beginning to separate from the shell on the trees, and many did separate upon drying in the laboratory. Shell appeared mature, and kernel was solid.

October 3, 1939.—Shucks completely separated from the shell. Kernels considered mature, since they had no attachment with the shuck or the tree.

The samples were collected from the trees in the blocks according to date of treatment. The blocks consisted of 10 trees except in one instance where there were only 8 trees. At each sampling date, shoots [4] bearing 4 nuts were taken at random from the east and west sides of each tree. The nuts [5] were then sampled separately from the shoots, and each sample was weighed in the field. The samples were dried at 65° C. to constant weight for moisture determination; after drying they were ground in a Wiley mill to pass a 40-mesh sieve. The ground material was placed in large mason jars and mixed by shaking for 15 minutes in a rotary shaking apparatus.

The total content of ash, nitrogen, phosphorus, calcium, magnesium, and potassium in the nuts and shoots was determined. The standard methods (*1*) were used for nitrogen, phosphorus, and calcium. The method described by Hillebrand and Lundell (*9*) was used for magnesium, and that of Scott (*13*) for potassium.

## RESULTS OF ANALYSES

The analytical data show that marked changes in the composition of the nuts and shoots occurred between the dates of sampling. It was recognized that more than one season's results would be required to interpret adequately the variations due to dates of treatment.

[4] The term "shoot" as used in this paper includes the leaves borne on the shoot also.
[5] The term "nut" as used in this paper includes the shuck, shell, and kernel.

However, it appeared that the samples from trees differing only in dates of treatment might be considered as valid replicates for determining the significance of the changes occurring between the dates of sampling. All the factors causing variance could not be separated from the data, but it was possible to determine the true error plus the interaction, if any, between dates of sampling and treatments. Thus, with these reservations, the data were subjected to the analysis of variance as described by Snedecor (*18*), and the means for the eight determinations for each date of sampling are reported. The interaction thus calculated was used for the error term, and in all probability it is too high rather than too low.

The data for the mean percentage composition of the dry matter are given in table 1. These data converted to grams of constituents per shoot and per four nuts[6] are given in table 2. In many ways the actual amounts of mineral constituents show the changes involved in the process of the development of the nuts more clearly than does the percentage composition.

TABLE 1.—*Mean composition of pecan nuts during development and of their supporting shoots (dry-weight basis), 1939*

| Part sampled and date of sampling | Dry matter | Ash | Nitrogen | Calcium (CaO) | Magnesium (MgO) | Potassium ($K_2O$) | Phosphorus ($P_2O_5$) |
|---|---|---|---|---|---|---|---|
| Nuts: [1] | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| July 15 | 25.62 | 7.59 | 1.295 | 2.146 | 0.538 | 1.654 | 0.470 |
| August 15 | 26.12 | 5.59 | .988 | 1.156 | .325 | 1.271 | .370 |
| September 15 | 32.09 | 4.98 | 1.042 | .731 | .225 | 1.309 | .390 |
| October 3 | 36.55 | 4.99 | 1.117 | .690 | .226 | 1.318 | .409 |
| Least significant difference ($P=0.05$) | .85 | .17 | .050 | .042 | .019 | .154 | .015 |
| Least significant difference ($P=0.01$) | 1.16 | .23 | .068 | .057 | .025 | .209 | .021 |
| Shoots: [2] | | | | | | | |
| July 15 | 43.37 | 8.49 | 2.013 | 3.088 | .544 | 1.004 | .310 |
| August 15 | 43.62 | 8.75 | 2.029 | 3.385 | .518 | 1.117 | .300 |
| September 15 | 44.55 | 9.25 | 1.830 | 3.589 | .521 | .996 | .328 |
| October 3 | 43.18 | 8.99 | 1.732 | 3.526 | .479 | .760 | .313 |
| Least significant difference ($P=0.05$) | [3] | .21 | .069 | .087 | .023 | .116 | .019 |
| Least significant difference ($P=0.01$) | ------- | .28 | .093 | .119 | .031 | .159 | .027 |

[1] The entire fruit including shuck, shell, and kernel.
[2] The terminal growth including 4 leaves.
[3] Not significant according to $F$ test.

TABLE 2.—*Mean constituents*[1] *in pecan nuts during their development and in the supporting shoots, 1939*

| Part sampled and date of sampling | Dry matter | Ash | Nitrogen | Calcium (CaO) | Magnesium (MgO) | Potassium ($K_2O$) | Phosphorus ($P_2O_5$) |
|---|---|---|---|---|---|---|---|
| Nuts: [2] | *Grams* | *Grams* | *Gram* | *Gram* | *Gram* | *Gram* | *Gram* |
| July 15 | 4.33 | 0.3283 | 0.0560 | 0.0928 | 0.0233 | 0.0716 | 0.0204 |
| August 15 | 13.85 | .7745 | .1367 | .1601 | .0450 | .1759 | .0512 |
| September 15 | 22.76 | 1.1332 | .2371 | .1662 | .0512 | .2977 | .0888 |
| October 3 | 25.39 | 1.2664 | .2833 | .1749 | .0574 | .3344 | .1038 |
| Least significant difference ($P=0.05$) | .62 | .0499 | .0127 | .0087 | .0068 | .0237 | .0053 |
| Least significant difference ($P=0.01$) | .85 | .0679 | .0173 | .0119 | .0011 | .0323 | .0073 |
| Shoots: [3] | | | | | | | |
| July 15 | 7.75 | .6581 | .1560 | .2394 | .0421 | .0779 | .0240 |
| August 15 | 8.21 | .7185 | .1665 | .2780 | .0426 | .0919 | .0246 |
| September 15 | 7.38 | .6811 | .1349 | .2643 | .0385 | .0734 | .0242 |
| October 3 | 6.69 | .6009 | .1159 | .2359 | .0321 | .0507 | .0209 |
| Least significant difference ($P=0.05$) | .44 | .0397 | .0062 | .0164 | .0031 | .0106 | .0015 |
| Least significant difference ($P=0.01$) | .59 | .0541 | .0085 | .0224 | .0042 | .0144 | .0021 |

[1] The calculated amounts of constituents for 4 nuts and for their supporting shoot.
[2] 4 entire fruits including shuck, shell, and kernel.
[3] The terminal growth including 4 leaves.

[6] Units of 4 nuts were used rather than 1, since all the shoots supported 4 nuts.

In order to evaluate properly the importance of the changes shown by the data, the stage of development of the nuts must be kept in mind. The first period, July 15 to August 15, was one of very active growth during which the nuts made from one-half to two-thirds of their total increase in size. Differentiation of the shuck, shell, and kernel took place, the shells became hard, and the seed coat of the kernel developed and was full of endosperm, but little or no discernible embryo formation occurred. This period was characterized by no significant change in the percentage of dry matter in the nuts, whereas the actual dry weight per nut increased at a highly significant rate. A highly significant decrease in the percentage of nitrogen, calcium, magnesium, potassium, and phosphorus in the dry matter occurred, while the actual amount of these elements per unit of nut increased in highly significant quantities, these results showing that movement of the elements into the nuts was taking place, but at a slower rate than the accumulation of actual dry weight in the nuts.

During this first period the percentage of dry matter did not change significantly in the shoots, whereas there was a significant increase in the dry weight per shoot. There was no significant change in the percentage of nitrogen, potassium, or phosphorus, a significant increase in the percentage of ash, a highly significant increase in the percentage of calcium, and a significant decrease in the percentage of magnesium in the dry matter. Because of a significant increase in the dry weight per shoot, there was a significant increase in the amount of potassium per shoot and a highly significant increase in ash, nitrogen, and calcium while the magnesium and phosphorus did not change significantly.

There was no evidence of a withdrawal of any of these constituents from the shoots by the nuts, and the ash, nitrogen, calcium, and potassium accumulated in the shoots at a more rapid rate than they were being used by the nuts.

During the second period, August 15 to September 15, the nuts increased very little in size, the shuck reached the point of partial separation from the shell, the shell appeared mature, and the kernal became a solid mass of fleshy material.

This period was characterized by highly significant increases in the percentage of dry matter and in the dry weight per unit of nut. These increases were accompanied by a significant increase in the percentage of nitrogen and phosphorus, no significant change in the percentage of potassium, but a highly significant decrease in the percentage of ash, calcium, and magnesium. In actual amount of these constituents per unit of nut, calcium was the only constituent that did not accumulate in the nuts in highly significant quantities. The very rapid intake of nitrogen, phosphorus, magnesium, and potassium into the nuts during this period is of particular interest when considered in the light of the studies of Thor and Smith (*20*, *21*), who found that during kernel development such organic constituents as oil, protein, and acid-hydrolyzable polysaccharides accumulated in the nuts at a very rapid rate. The results indicate a direct correlation between the movement of these organic constituents and that of nitrogen, phosphorus, magnesium, and potassium into the nuts. The very slow rate of intake of calcium into the nuts during this second period

indicates that this element accumulates in the nut before kernel development.

During this period there was no significant change in the percentage of dry matter in the shoots, but there was a highly significant decrease in the dry weight per shoot. There was a highly significant increase in the percentage of ash, calcium, and phosphorus in the dry matter, a highly significant decrease in nitrogen, a significant decrease in potassium, and no significant change in the magnesium. In units per shoot there was a highly significant decrease in nitrogen and potassium, a significant decrease in magnesium, and no significant change in ash, calcium, and phosphorus. These results indicate that the nuts were withdrawing from the shoots all the constituents except calcium and phosphorus at a more rapid rate than they were being replaced.

During the third period, September 15 to October 3, the nuts were filled with kernels which became mature. On the whole, the changes noted for this period were a continuation of those mentioned for the second period. Nitrogen, phosphorus, magnesium, and potassium continued to move into the nut in highly significant quantities, but at a slightly slower rate than during the previous period. Calcium moved into the nuts at a slightly higher rate during this period than during the preceding one.

As in the nuts, the changes in the constituents of the shoots were largely a continuation of those noted in the preceding period, except that during this period the decrease in phosphorus was highly significant, whereas earlier it was not significant. It is interesting to note that during the period of nut filling the actual amounts of the mineral constituents in the shoots decreased at the same time that they were being accumulated in the nuts.

As stated previously, the data on the nuts as reported in this paper were obtained from the whole nut, including the shuck, shell, and kernel. It would have been impossible to separate the two earliest samples into these different parts because differentiation had not taken place. In the last two samples this separation could have been made, and had this been done no doubt a clearer knowledge of the changes associated with the development of the kernel would have been obtained. At the final harvest, samples of nuts were obtained from the trees according to dates of treatment, and separate analyses were made on a composite sample of the shells and kernels from each treatment. These data are presented in table 3.

The percentage of nitrogen in the kernel was highest, followed in order by potassium, phosphorus, magnesium, and calcium. Near maturity, October 3, the percentage of potassium in the whole nut was highest, followed by nitrogen, calcium, phosphorus, and magnesium. These changes in the order of the elements in the kernel as compared with those in the whole nut show that nitrogen and magnesium are deposited in the kernel to a relatively greater degree than are the other elements. This would indicate that a systematic study of the mineral constituents of the shuck, shell, and kernel from the time of differentiation of the tissues to maturity might reveal some interesting relations.

TABLE 3.—*Mean composition*[1] *(dry-weight basis) of mature pecan kernels and shells, 1939*

| Part | Ash | Nitrogen | Calcium (CaO) | Magnesium (MgO) | Potassium ($K_2O$) | Phosphorus ($P_2O_5$) | Oil |
|---|---|---|---|---|---|---|---|
| | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Kernel | 1.67±0.07 | 1.684±0.082 | 0.107±0.008 | 0.248±0.006 | 0.946±0.057 | 0.318±0.012 | 73.1±1.1 |
| Shell | 2.27± .06 | .293± .025 | 1.032± .043 | .068± .007 | .480± .027 | .061± .009 | ---------- |

[1] 8 samples collected at harvest.

## DISCUSSION

The data in this report and that of Thor and Smith (*20*, *21*) on the changes in organic constituents of the pecan during development bring out some interesting correlations. Nitrogen was determined in both of these studies, and there is a strikingly close similarity in the amount found in the nuts at different stages of development and in the trend of the changes recorded. For example, in the Texas study (*21*) the nitrogen content of the nuts was 1.15 and 0.96 percent on July 14 and August 14, respectively, and in this study it was 1.295 and 0.988 percent on July 15 and August 15, respectively. As these were the only two calendar dates that coincided other direct comparisons cannot be made; but it is evident from these data that the course of change in the nitrogen content was similar in the two studies. So close an agreement between studies conducted under such different environmental conditions is rather surprising.

It was pointed out earlier (p. 302) that the period of rapid intake of nitrogen, phosphorus, magnesium, and potassium into the nuts as shown in this study coincides with the period of rapid accumulation of such organic constitutents as oil, protein, and acid-hydrolyzable polysaccharides in the nuts as shown by the study in Texas. In both studies, this period coincided with the period of rapid kernel development. It is indicated that the four elements named above play an important role in the assimilation and storage of the organic constituents that make up the greater part of the pecan kernel. It is conceivable that a deficiency of any of these elements or a lack of balance between them at the time of kernel development might result in poorly filled nuts.

This study shows that the greater part of the calcium in the nuts accumulates there during the period when the nuts are growing in size and that relatively small amounts move into the nuts during the filling period. This would indicate that calcium is more important in the formation of structural tissues than it is in the formation of the kernels. The content of calcium stored in the kernel supports this theory.

The reduction in weight of the pecan shoots and the decrease of minerals in the shoots during the filling of pecans may have an important bearing on the tendency of the pecan to bear biennially. The pecan matures its nuts late in the season, thus leaving very little time for the tree to build up reserves for the next season. This makes it very important that the trees be supplied with sufficient minerals in the proper balance so that the mineral and organic reserves will

not be too greatly depleted in the shoots by a heavy nut crop. A quick recovery of the organic reserves necessary for strong shoot development in the spring may be associated with and dependent on the quantity and balance of the mineral reserves after the harvest of a heavy crop.

## SUMMARY

During the growing season eight blocks of pecan trees were sampled on four different dates representing different stages in the development of pecan nuts. Dry matter, ash, nitrogen, calcium, magnesium, potassium, and phosphorus were determined in the nuts and shoots. Data on the composition are reported for different dates, and the changes as related to the stage of the development of the nuts are discussed.

The percentage composition of the pecan nut and of its supporting shoot is influenced to a high degree by the stage of development of the nut. In the last month of the period of growth in size, the actual dry weight of the nut increased at such a rapid rate that the percentage of mineral constituents in the dry matter showed highly significant decreases even though they continued to accumulate in the nut in highly significant quantity on the basis of units per nut. The shoot increased in dry weight and in the content per shoot of all the minerals except magnesium and phosphorus.

In the second and third periods studied, during which the kernel of the pecan developed and filled, nitrogen, potassium, magnesium, and phosphorus accumulated in the nut at rates exceeding the accumulation of dry matter even though the dry weight of the nut itself increased at a rapid rate. Calcium accumulated in the nut in very small quantities. The shoots lost significant quantities of all the minerals.

Data on the composition of pecan kernels and shells show that calcium is deposited largely in the shell while nitrogen, potassium, phosphorus, and magnesium are deposited to a greater extent in the kernel. Composition of the whole nut does not give a true picture of the changes taking place in the nut during kernel development.

The unit basis of expressing the results of analyses such as are reported here was found to be more satisfactory than expressing the results on a percentage basis.

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# A POMOLOGICAL AND CYTOLOGICAL STUDY OF A RUSSETED SPORT OF THE STARK APPLE[1]

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## INTRODUCTION

Many of the present day varieties of horticultural fruits owe their origin to bud sports which can readily be propagated by asexual methods (*9*).[2] The hybrid nature of these fruits, particularly in the pome and citrus groups, precludes a detailed genetical analysis of such somatic segregation, with the result that comparatively little can be stated with certainty regarding their mechanism of origin. The lack of genetical data and the small size of the chromosomes also prohibit the recognition of cytological changes other than the gain or loss of whole chromosomes. Duplication or deletion of chromosome segments would not be detected. Jones (*4*, *5*, *6*), on the other hand, using marker genes in maize endosperm, has demonstrated conclusively that chromosome aberrations can lead to atypical growths as well as color mosaics, both types of which are frequent in pome and citrus fruits.

A recent cytological survey of some bud sports in apple (*Malus pumila* Mill.) has indicated that changes of sufficient magnitude can be pursued to advantage in elucidating the mechanism of bud-sport origin. A single variable bud sport of the Stark apple provides the source of material for this study.

## MATERIAL AND METHODS

Incident to the junior author's study of bud sports in Michigan, four solid-red limb variants were found, one each in orchards near Kibbie, Lacota, Fennville, and South Haven, and one russeted sport in the George B. Meechum orchard near Fennville. The present article deals with this russet mutation. A comparatively large part of the sporting portion of the parent tree had been top-grafted at the time it was brought to the junior author's attention in 1927, so that, while cions could be obtained, observations as to the range of variation in character of fruit on the sporting limb could not be comprehensive.

Cions of the russet sport were obtained in 1927 and designated as Stark strain No. 287. Some were set as top-grafts in trees of bearing age in the Graham Experiment Station orchard near Grand Rapids. Others were set on small nursery seedling stock. Two of these nursery-propagated trees were planted in the orchard in the spring of 1932. The top-grafts bore their first fruits in 1933 and fruited regularly thereafter. The first crop of any considerable size produced by the orchard trees was in 1939.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 315.

For the cytological study, material from the nursery-propagated trees was collected in late February 1941 and forced into bud in the greenhouse. These branches had been labeled the preceding fall prior to harvest, and included those of the russet sport, and single whole branches of what appeared to be partial and complete reversions to the original Stark type. Branches from a normal Stark were included for comparison. Fixation was in 3:1 alcohol-propionic acid; the smears were made by the usual acetocarmine method.

## POMOLOGICAL STUDY

### CHARACTERISTICS OF THE STARK APPLE

The Stark apple, said to have originated in Ohio, has been in cultivation for three-quarters of a century *(1, v. 1, p. 317)*. Though never attaining a commanding position in any important commercial producing district, it has been rather widely planted and is recognized as a more or less standard late-winter variety in the Northeastern States. Many orchards contain trees of this variety numbering in the hundreds.

Characteristically, its fruits have a greenish-yellow ground color and are striped, splashed, and blushed with a rather dull red. Where shaded, the ground color predominates; where well exposed to the sun, the overlying color predominates. Under favorable growing conditions, the skin is smooth and glossy. Under conditions of relatively high humidity, or when certain spray materials are used, the cuticular layer is more or less broken and roughened and a portion of the surface presents a slightly russeted appearance. This normal russeting, if such it may be called, is only partial and seldom covers more than a quarter or a third of the surface. It is thin and generally irregularly distributed over the surface of the fruit.

Though observation indicates that Stark is less given to producing limb sports than many other apple varieties, a number have been reported. In 1932, Shamel and Pomeroy *(9)* listed seven solid-red or high-color variants in the fruits.

### CHARACTERISTICS OF RUSSET STRAIN NO. 287

Fruits of the russet bud sport borne by the top grafts in 1933–37 were rather uniformly of normal size and shape for the Stark variety, but differed from the normal in being fairly completely covered with an even, thin coat of russet. Irregular patches or areas of the normal glossy skin were present in a small percentage of the fruits. In the year 1938, these top-grafts again bore a medium crop of fruit, but only two species were like those borne the 5 preceding years. The remainder were of more or less normal shape for Stark, but in size ranged from only one-third to two-thirds normal and were covered with a thick, heavy, coarse russet. The smallest specimens were the most heavily and coarsely russeted and they were also rather deeply cracked, there being a clear correlation between the heaviness of russeting, the depth of the cracking, and the progressively smaller size of the fruits (figs. 1 and 2). The crops borne by these top-grafts in the 1939–42 period were like those of the 1933–37 period, except that an occasional fruit showed a tendency to be heavily and coarsely russeted and more or less cracked. Detailed russeting records of the 1939 and 1940 crops of these top-grafts are presented in table 1.

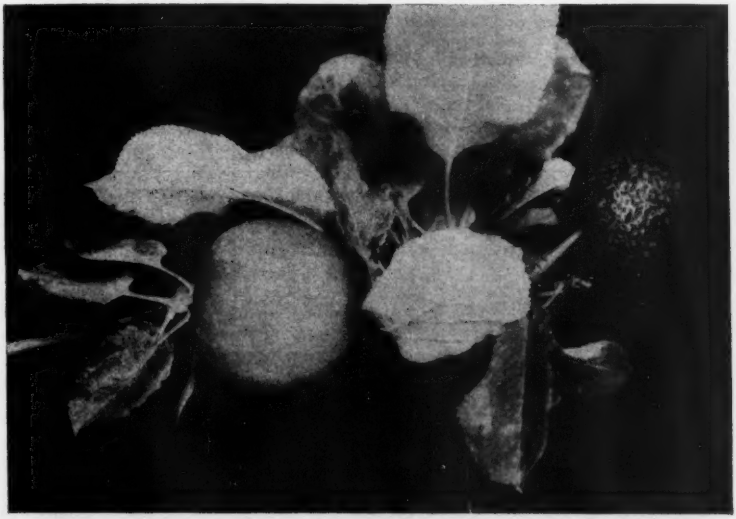

FIGURE 1.—The apple on the left is a thinly and evenly russeted fruit, typical of Stark strain No. 287. The specimen on the right is a typical, small-sized, coarsely russeted and somewhat cracked variant from strain No. 287.

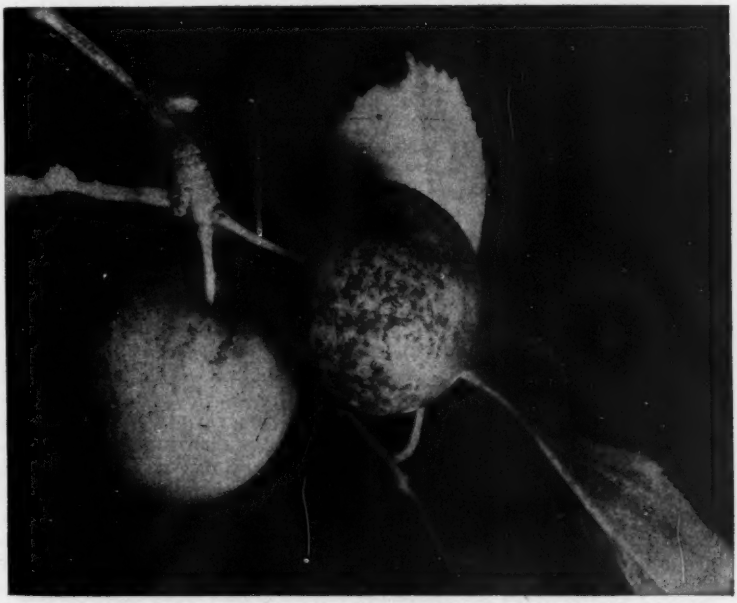

FIGURE 2.—Two fruits borne by a single spur of Stark strain No. 287. The one on the left shows a slight and the one on the right a marked tendency toward coarse russeting and cracking, typical of the entire 1938 crop on the top-grafts of this strain and appearing occasionally in the 1939–42 crops.

TABLE 1.—*Frequency distribution table showing numbers of fruits of Stark strain No. 287, top-grafts and orchard trees, with varying percentages of their surface covered with russet, seasons of 1939–41*

| Location in orchard | Year | Percentage of surface covered by russet— | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1–25 | | 25–50 | | 50–75 | | 75–100 | |
| | | *Number* | *Percent* | *Number* | *Percent* | *Number* | *Percent* | *Number* | *Percent* |
| Top-graft | 1939 | 8 | 4 | 20 | 10 | 45 | 24 | 118 | 62 |
| | 1940 | 0 | 0 | 9 | 7 | 51 | 43 | 62 | 50 |
| Tree B-19 | 1939 | 17 | 12 | 23 | 17 | 28 | 21 | 67 | 50 |
| | 1940 | 0 | 0 | 23 | 12 | 63 | 34 | 99 | 54 |
| | 1941 | 12 | 2 | 3 | 1 | 32 | 6 | 481 | 91 |
| Tree B-20 | 1939 | 100 | 31 | 59 | 19 | 59 | 19 | 97 | 31 |
| | 1940 | 0 | 0 | 4 | 4 | 22 | 20 | 83 | 76 |
| | 1941 | 14 | 2 | 8 | 1 | 123 | ..... | 487 | 77 |

The fruits borne by the 2 orchard trees each year during the 1939–42 period were much like those borne by the top-grafts during the same period. Most specimens were of normal size and shape for the Stark variety and were thinly and evenly russeted. However, distributed irregularly throughout the trees were specimens somewhat cracked (figs. 1 and 2). In 1941 each of the 1,182 fruits borne by the 2 orchard trees was weighed and the amount and density of its russeting estimated, the estimate being largely on the basis of the percentage of its surface that was covered with russet. The correlation coefficient between fruit weight and amount of russeting was $0.364 \pm 0.017$. The distribution of the heavily russeted fruits was by spur rather than by branch. That is, no whole branches were found that bore the heavily russeted fruits one year, and necessarily produced the same kind of fruits the following year. Occasionally when two fruits were borne by a single spur one would be thinly and evenly russeted, while the other was heavily russeted, as shown in figure 2.

One small branch, springing from a lower horizontal limb of one of the two orchard trees of this strain, was noted in 1939 as producing smooth-skinned, glossy fruits exclusively, thus showing complete reversion to the normal Stark parent. This reverting branch, shown in figure 3, has continued to bear smooth fruits.

## CYTOLOGICAL STUDY

The Stark apple possesses a diploid number (fig. 4, *A*, *B*, and *C*), a chromosome number different from that of the usual apple varieties which show multiples of a haploid number 17. This agrees with previous findings of Newcomer (*7*). Roscoe (*8*), however, has reported a diploid number of 51, making this variety a triploid. In view of the fact that a diploid number of 42 is aberrant for members of the Pomoideae, the preparations were carefully scrutinized and several additional strains of Stark were examined, but in all instances the same chromosome count of 42 was found. This suggests that possibly 2 cytologically different strains of the Stark apple exist, but since all Stark trees undoubtedly stem from the same origin, this seems unlikely.

Univalents were frequent at metaphase I, but they were usually incorporated into one or the other of the nuclei before telophase reorganization (fig. 4, *A*). Chromatin bridges at anaphase indicated the presence of inversions. Diplotene (fig. 4, *C*) was clear but difficult

to analyze for multiple associations. Trivalents were present at metaphase, with six the maximum recorded.

The russeted sport possessed a complement of 51 chromosomes (figs. 5, *A* and *B*, and 4, *D*, *E*, and *F*). Meiotic configurations were very similar to those described by Roscoe (*8*), with trivalents and quadrivalents frequent. Multiple associations were observable at diplotene (fig. 4, *D*), but at diakinesis (fig. 4, *E*) the looseness of pairing made this uncertain. Interesting to note was the stretched condition of the nucleolus at diplotene and diakinesis (fig. 4, *D* and *E*), a condition also noted before pressure was applied to the cover glass in making the smears. It would appear that a repulsion of bivalents attached

FIGURE 3.—Branch on one of the trees of Stark strain No. 287 that shows a complete reversion to the normal Stark type with smooth, glossy skin. The spur on the left is bearing one typically russeted fruit and one that has varied in the direction of rough, coarse russeting. The point where reversion has occurred is just to the right of the spur on the left that is bearing two fruits.

by their nucleolar constrictions leads to a stretching of the nucleolus from a sphere to a spindle-shaped body. A comparison of cells in comparable stages of prophase shows that the nucleolus is much larger in the russet sport than in the normal Stark strain.

The single branch showing a complete reversion to the parental type (fig. 3) gave a cytological picture similar in every detail to that of its progenitor. Again the chromosome number was 42.

Several branches showing what was assumed to be a partial reversion to the parental type were selected for study. With the exception of one, which was more decidedly in the direction of the original Stark in size, shape, and degree of russeting, all possessed 51 chromosomes. The exceptional branch showed a somatic complement of 46 chromosomes in the cells of the tapetum. Counts of 45 and 47 were made but the majority of them revealed 46 (fig. 5, *C*, *D*, and *E*),

particularly when anaphase figures were utilized. Occasional cells from those flower buds toward the base of the branch had 51 chromosomes, indicating that the basal portion was the place of origin of this mutant branch and that a mixture of cells was present. There was no change in the external morphology of the stem and leaves which pointed to an altered cytological condition, although it is quite possible that the polyploid condition of the apple permits a cytological change without visible morphological expression.

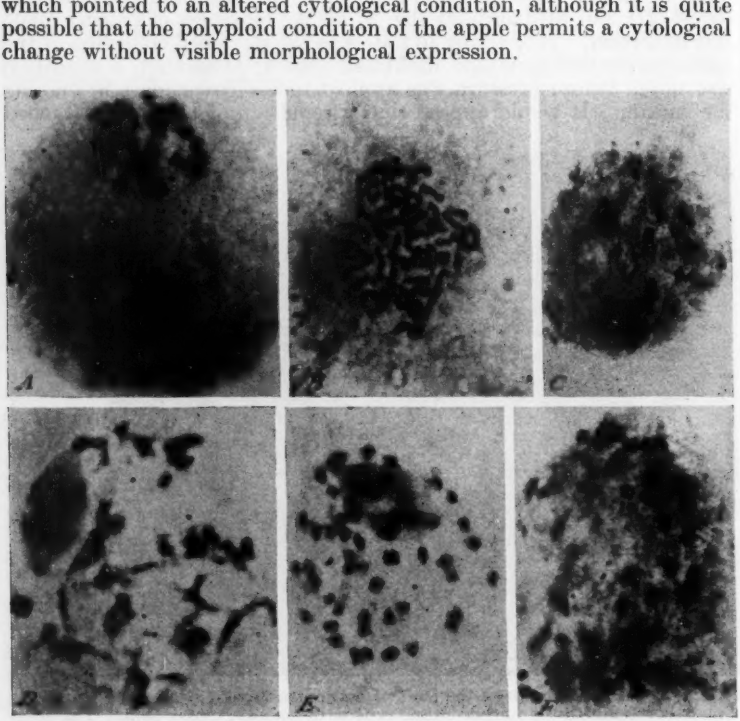


FIGURE 4.—Normal Stark: Anaphase I (*A*), somatic metaphase from tapetal tissue (*B*), diplotene (*C*). Russet bud sport: Diplotene (*D*), diakinesis (*E*), anaphase I (*F*). × 1,000.

## DISCUSSION

In a number of respects Stark strain No. 287 is like many bud sports of the apple that have been under study at the Michigan station, e. g., (1) it has remained sufficiently true to type to be classified as a strain or variety distinct from the parent, and (2) it has yielded one or more complete reversions to the parent type. In one respect it has deviated from the usual bud mutant pattern, viz., it has in turn yielded "sports," still more extreme, in the same direction in which it deviated from the parent form. In this latter instance the sporting apparently has taken place in the flower bud primordia, rather than in the leaf buds. This is evidenced by the irregular occurrence and distribution of the coarsely russeted variants and also by their "wholesale" occurrence on the top-grafts in 1938, and in 1938 only, of the 10 years that fruits have been borne by the top-grafts and

trees under study. Presumably the wholesale sporting of that year was due indirectly to some factor or combination of factors of the environment of that season (1938) or of the preceding season while flower bud primorida were being differentiated or developed. That it cannot be attributed to any direct influence of 1938 conditions on russeting per se is indicated by the fact that the mean percentage of russeting (i. e., percentage of surface of fruits covered by russet) on 5,226 fruits of a large 50-year-old Golden Russet tree growing within 200

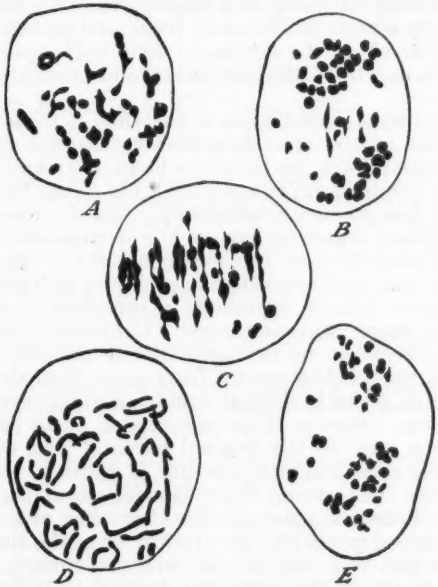


FIGURE 5.—Russet bud sport: Metaphase I (*A*), anaphase I (*B*). Forty-six-chromosome reversion: Metaphase I (*C*), somatic metaphase from tapetal tissue (*D*), anaphase I (*E*). × 1,350.

yards of the top-grafts of strain No. 287 was 9 in 1938, and for its 16,590 fruits in 1939 it was 18. Climatic conditions in 1939 were therefore twice as favorable for russeting as in 1938, when the russeting of the fruits on the Stark strain No. 287 top-grafts was so greatly accentuated.

In view of Jones' (*4*, *5*, *6*) clarification of atypical growths and color mosaics in maize endosperm, there is sufficient reason for assuming that chromosomal aberrations lead to many of the bud sports arising constantly in horticultural plants. Huskins' (*3*) summary, which indicates that most of the dominant and some of the recessive mutations, so-called, found in polyploids are attributable to chromosomal aberrations affecting appreciable regions of the chromosome, or even whole chromosomes, lends additional support to the belief that bud sports in apples are the result of chromosomal changes since the apple is a derived polyploid, and mutations or bud sports arising somatically must be dominant to be recognized. The present lack

of evidence can no doubt be traced to the inadequacy of the apple as a subject for cytological studies.

The cytological study reported here suggests that the russet sport No. 287 arose from the normal Stark by an addition of nine chromosomes, and that this increase brought with it an unstable genetic condition. The mechanism for this chromosomal increase is not understood, but the instability of the genotype is expressed by the varying degrees of russeting both between seasons and within and between spurs and branches, and also by the varying chromosomal constitution of different parts of a single tree. The russeting variability is strikingly similar to the color, form, and maturity variability found by Gardner (*2*) in the hetero-chimeric apple sport "Graham," though in the latter the variations when once produced, appear to be more stable.

The 51-chromosome russet sport is therefore a triploid, but not a balanced triploid of the sort described by Roscoe (*8*). The 42-chromosome normal Stark, on the other hand, is an aneuploid in that its chromosome complement is not a multiple of the haploid number 17. Assuming, however, as a possibility, that the russet bud sport arose as a dominant mutation from a 51-chromosome Stark such as described by Roscoe, it then becomes necessary to account for the origin of the 42- and 46-chromosome branches as well as the fact that the 42-chromosome branch represents a complete reversion to the normal Stark in shape, size, and color. It appears likely, although still difficult to visualize in the light of our present cytogenetical knowledge, that the 51-chromosome form arose from the 42-chromosome normal Stark, possibly through somatic nondisjunction, and that the 42-chromosome reversion is not a reversion at all but merely the result of carrying some of the original parental cells over from the source tree (the normal Stark) at the time of grafting. The inclusion of 51-chromosome cells among those showing 46 at the base of the partial reversion branch suggests further that a loss of 5 chromosomes occurred in the apical meristem just prior to, or at the time of, branch initiation. The fact that this partial reversion branch appeared no different from others on the same tree seemed to indicate that the chromosome loss affected the growth characteristics but little.

It is evident in any event that the russet Stark No. 287 is a complex chromosomal chimera, and that by the outcropping of various tissues having different chromosome numbers, the establishment of variant branches has been brought about. The origin of the variant tissues probably lies in an unstable somatogenesis, conditioned perhaps by a gene unbalance.

## SUMMARY

A russeted bud sport of the Stark apple is described. The russeting was generally thin and even, but showed considerable variability within trees and between seasons. Coarse russeting was correlated with deep cracking and a decreased weight of the fruit.

The bud sport showed a tendency to revert to normal as well as to "mutate" toward a more extreme form of russeting. With the exception of one "normal" branch, the other "mutations" appeared to be conditioned by environmental factors.

The bud sport revealed a diploid chromosome number of 51, while the normal Stark showed a diploid number of 42. The branch show-

ing complete reversion to normal possessed a chromosome number of 42, and one branch showing partial reversion had a chromosome number of 46. Other "partially reverted" branches showed no alterations in chromosome number.

It was suggested that the 51-chromosome sport arose from the 42-chromosome Stark (which is an aneuploid) by an addition of chromosomes, possibly through somatic nondisjunction, and that the 46-chromosome branch resulted from a loss of chromosomes in the branch meristem. The 42-chromosome completely reverted branch may have resulted from active cells carried over from the parental tree through the graft.

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# SURVIVAL ON GRASS PLOTS OF EGGS AND LARVAE OF THE STOMACH WORM, HAEMONCHUS CONTORTUS[1]

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## INTRODUCTION

The large stomach worm (*Haemonchus contortus*), a destructive parasite of sheep and other ruminants, is acquired by a susceptible animal through ingestion of infective larvae with the feed. Control of this parasite depends on removing the adult worms by suitable anthelmintics or preventing or reducing infection of susceptible animals by improved pasture management or a combination of the two methods. The type of pasture management to be used depends on the extent of survival of infective larvae under natural conditions.

## REVIEW OF LITERATURE

Since Ransom (*10*)[2] worked out the life history of *Haemonchus contortus*, a number of studies have been conducted to determine how long eggs or larvae of this species would survive on pasture. Ransom found that larvae kept out of doors for 3 months during the winter showed little reduction in the number of contained food granules. During his experiments, the larvae were frozen for about one-third of the time. Ransom (*11*) also found that "pastures on which infested sheep have grazed will not become free from infestation with the twisted wire worms after remaining empty from October 25 to June 16, climatic conditions being similar to those of Washington, D. C." This writer (*12*) also reported that pastures kept free of ruminants for a year are practically free of stomach worm infection. He considered that infective larvae of *H. contortus* were slightly affected by dryness and freezing. Veglia (*16*), on the other hand, indicated that infective larvae were unable to withstand natural climatic conditions in South Africa, and unless protected by shade or subjected to rainfall many, and often all larvae, were dead after an exposure of less than 1 day. Mönnig (*9*) showed that infective larvae of *H. contortus* died in less than 3 months during dry weather in South Africa.

Dikmans and Andrews (*7*) allowed lambs in the spring to graze on pasture that had been unoccupied during the winter. The lambs became infected with *H. contortus* as well as with some other nematodes. Boughton and Hardy (*3, 4, 5, 6*) stated that in Texas infective larvae of *H. contortus* survived on pastures for 22 months, but after 31 months the lambs allowed to graze on a pasture previously infested remained free from infection. Griffiths (*8*), using uninfected lambs for testing the survival of larvae on paddocks, found that *H. contortus* larvae did not survive from October to May on Montreal

[1] Received for publication February 8, 1943.

[2] Numbers in parentheses refer to Literature Cited, p. 323.

Island, Canada. Swales (*14*) confirmed Griffiths' work and found that in eastern Canada larvae of *H. contortus* apparently did not survive over a period lasting from fall (August to November) until the following June. Baker (*1, 2*) stated that at Ithaca, N. Y., worm-free lambs became infected with *H. contortus* and other worms when allowed to graze on a pasture 12 months after heavily infested sheep had grazed on it until all grass was eaten down to the crowns. This author succeeded in infecting two lambs by allowing them to graze on this pasture 21 months later. Taylor (*15*), in reporting experiments carried out in 28-inch-square boxes subdivided into 4-inch-square grass plots and inoculated September 6, 1937, with "ovine trichostrongylid larvae," stated that death of the larvae was constant and rapid. Viable larvae were found as late as 133 days after the boxes were prepared. The identity of the larvae was not given.

The following is a summary of the survival periods, as determined by the various investigators, of the preparasitic stages of *H. contortus:* 22 months but not 31 months by Boughton and Hardy; at least 21 months by Baker; at least 8 months by Ransom—he believed that pastures would be practically free of the infective larvae after 1 year; over the winter by Dikmans and Andrews; less than over the winter by Griffiths and by Swales. Boughton and Hardy found a total of only three adult *H. contortus* in two lambs that had grazed on a pasture contaminated 22 months previously by sheep infected with the nematode in question. The number of *H. contortus* surviving in Baker's experiments is not known as the lambs used in the test were not removed from the pasture before the worms acquired had time to grow to maturity, recontaminate the pasture, and repeat the life cycle. The fact that Ransom was unable to maintain lambs free from *H. contortus* by the method reported in his paper throws some doubt on the conclusions drawn in connection with his survival studies.

An examination of reports of previous studies makes it possible to conclude only that larvae of *H. contortus* will survive over the winter under certain conditions, but the number of larvae surviving and capable of causing infection at any given time after pasture contamination is not definitely known. Therefore, the studies reported in this paper were made to ascertain whether enough larvae on a contaminated pasture die during the fall and winter to make the pasture safe for sheep the following spring. The work was carried on from August 1936 to March 1938 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

## MATERIALS AND METHODS

In the present experiments 4-foot-square grass plots, each divided into 2-foot-square subplots, were used. The plots were separated by a space about 3 feet wide. The soil was sandy loam. The plots were sown with a mixture of bluegrass, meadow fescue, timothy, redtop, Italian rye, white clover, and alsike. Certain other plants appeared and were allowed to remain. As grasses were the most common plants present, the term "grass" is used as a designation for all the

vegetation. Except in the third experiment, the grass was cut at weekly intervals, if necessary, to keep it at a height of 4 inches. The grass trimmings were allowed to fall back on the plots, from which they disappeared shortly afterward.

The larvae and eggs used in the experiments were obtained from sheep infested either with *Haemonchus contortus* alone or with a combination of *H. contortus* and *Strongyloides papillosus*. Since the eggs and larvae of these two species can be easily distinguished, the slight contamination with *S. papillosus* may be ignored. No viable larvae of *S. papillosus* were found beyond the first week of exposure in any of the tests in which these larvae were originally present.

After the grass in the plots had grown to about 4 inches, feces from sheep infected with *H. contortus* were placed in the center of each subplot. In a week or more one of the subplots was examined for infective larvae, after which all the other subplots were examined at intervals of 1 or more weeks.

In examinations, the grass on a subplot was cut and the larvae washed from it. Larvae in feces, on the surface of the soil, and in the soil to a depth of 1 inch were recovered by means of the Baermann technique. In all cases the material studied—whether grass, feces, or soil—was taken from the entire subplot. The material was allowed to remain in the Baermann apparatus for 48 hours, and then about 30 cc. of water with any larvae present was withdrawn. If free-living nematodes were so numerous that they interfered with the counts of the larvae, the former were killed by means of hydrochloric acid by the method of Shorb (*13*). In each experiment, the difference between the average number of larvae in the control lots of feces and the number recovered on each date of collection represents the loss due to death of eggs and larvae as well as the loss due to manipulation of material. Loss of larvae in manipulation takes place in two ways. Some larvae migrate into the soil; others are lost in the Baermann apparatus, because recovery is never perfect.

Four experiments were conducted. In experiments 1 and 3, the parasitized feces were placed on the subplots in August and in experiments 2 and 4, in October.

## WEATHER DATA

Development and survival of *Haemonchus contortus* larvae depend to a large degree on meteorological conditions. Death of eggs and preinfective larvae is caused by cold or drying; death of infective larvae, largely by drying. A summary of the weather data during the period in which the experiments were in progress is shown in table 1. During the first period of the first two experiments the weather was unusually dry at the beginning; during the third and fourth experiments it was unusually wet at the beginning. At other times the weather was usually not far from normal for the region where the experiments were conducted.

TABLE 1.—*Summary of weather conditions* [1] *during experiments*

| Month | Temperature | | | | Mean relative humidity at— | | | Precipitation | |
|---|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Mean | Departure from normal (mean) | 8 a. m. | 12 m. | 8 p. m. | Total | Departure from normal |
| *1936* | ° *F.* | ° *F.* | ° *F.* | ° *F.* | *Percent* | *Percent* | *Percent* | *Inches* | *Inches* |
| August | 98 | 59 | 77.9 | +2.9 | 77 | 56 | 66 | 3.61 | −0.40 |
| September | 93 | 47 | 71.4 | +3.3 | 78 | 58 | 69 | 1.98 | −1.20 |
| October | 83 | 27 | 59.7 | +2.3 | 81 | 55 | 68 | 1.70 | −1.14 |
| November | 79 | 19 | 45.0 | −.2 | 68 | 48 | 54 | .76 | −1.61 |
| December | 65 | 14 | 39.0 | +3.2 | 78 | 62 | 68 | 5.28 | +1.91 |
| *1937* | | | | | | | | | |
| January | 76 | 29 | 43.8 | +10.4 | 79 | 75 | 73 | 7.83 | +4.28 |
| February | 64 | 20 | 36.6 | +1.3 | 70 | 54 | 56 | 3.33 | +.06 |
| March | 70 | 19 | 42.0 | −.6 | 68 | 47 | 50 | 1.50 | −2.25 |
| April | 93 | 34 | 53.9 | +.1 | 69 | 48 | 53 | 6.85 | +3.57 |
| May | 91 | 40 | 65.8 | −2.1 | 73 | 46 | 54 | 4.02 | +.32 |
| August | 97 | 62 | 77.8 | −2.8 | 86 | 62 | 72 | 6.70 | +2.69 |
| September | 94 | 45 | 65.9 | −2.2 | 85 | 55 | 69 | 1.76 | −1.58 |
| October | 82 | 31 | 55.3 | −2.1 | 82 | 57 | 69 | 8.81 | +5.97 |
| November | 75 | 23 | 46.6 | +1.4 | 73 | 46 | 56 | 3.88 | +1.51 |
| December | 72 | 19 | 37.0 | +.4 | 70 | 49 | 56 | .71 | −2.61 |
| *1938* | | | | | | | | | |
| January | 63 | 18 | 35.7 | +2.3 | 74 | 59 | 61 | 2.64 | −.91 |
| February | 71 | 21 | 40.9 | +5.6 | 72 | 56 | 59 | 2.37 | −.90 |
| March | 85 | 20 | 49.8 | +7.2 | 72 | 55 | 58 | 2.23 | −1.52 |

[1] Obtained from the monthly meteorological summary of the Washington, D. C., station of the Weather Bureau, U. S. Department of Commerce, located 14 miles from Beltsville, Md., where the experiments were carried out.

## EXPERIMENT 1. LARVAE PLACED ON GRASS PLOTS AUGUST 11, 1936

For the first experiment, 16 subplots were prepared on August 11, 1936, by placing 100 gm. of crushed fresh sheep feces in a pile in the center of each subplot. Four 100-gm. cultures prepared from the same lot of feces yielded an average of 50,000 larvae, a fact which showed that the infective material was heavily parasitized. During the first 2 months grass only was examined. For the remainder of the test, grass and soil to the depth of 1 inch were examined. The average number of larvae recovered from each subplot and the date of collection are recorded in table 2. This experiment showed a survival of larvae for 9 months during summer, fall, winter, and spring. There was a gradual reduction in the number of larvae recovered. Those obtained in April and May were all vacuolated, and it is probable that they were not infective. The sudden increase of larvae recovered in March was noticed in subsequent experiments and was probably due to the greater warmth, which attracted the larvae to the surface of the soil.

TABLE 2.—*Number of Haemonchus contortus larvae recovered from 16 subplots on which fresh sheep feces were placed August 11, 1936*

| Subplot No. | Date of collection | Larvae recovered [1] | Subplot No. | Date of collection | Larvae recovered [1] |
|---|---|---|---|---|---|
| | *1936* | *Number* | | *1937* | |
| 1a | Aug. 19 | 1,500 | 9d | Jan. 8 | 2 |
| 1b | Sept. 3 | 1,000 | 12c | Feb. 27 | 22 |
| 1c | Sept. 15 | 450 | 11a | March 24 | 278 |
| 1d | Sept. 22 | 397 | 11b | Apr. 3 | 108 |
| 12a | Oct. 15 | 700 | 11c | Apr. 20 | 3 |
| 9a | Oct. 28 | 96 | 11d | Apr. 27 | 28 |
| 12b | Dec. 16 | 133 | 12d | May 4 | 14 |
| 9b | Dec. 23 | 45 | | | |
| 9c | Dec. 30 | 46 | | | |

[1] From both grass and soil.

## EXPERIMENT 2. LARVAE PLACED ON GRASS PLOTS OCTOBER 26, 1936

A second experiment was carried out in the same manner as the one just described except that it was begun in October instead of August and, because of unavoidable circumstances, 2 months elapsed before the first examination of plots instead of 1 week as in the first experiment. Four cultures prepared from the lot of feces placed on the plots yielded an average of 100,000 larvae. The average number of larvae recovered from each of 13 subplots is shown in table 3. In the first experiment a few larvae were alive after 9 months, but in experiment 2 all were dead in 7 months. It is not known definitely whether the surviving larvae were always capable of producing infection as no tests to determine their infectivity were made, but it is probable that they were not infective even as late as April as all larvae recovered at that time were feeble and vacuolated. As the subplots examined on May 15, 1937, had no larvae the 3 remaining subplots were not examined and consequently are not included in the table.

TABLE 3.—*Number of Haemonchus contortus larvae recovered from 13 subplots on which fresh sheep feces were placed October 26, 1936*

| Subplot No. | Date of collection | Larvae recovered [1] | Subplot No. | Date of collection | Larvae recovered [1] |
|---|---|---|---|---|---|
| | *1936* | *Number* | | *1937* | *Number* |
| 5c | Dec. 21 | 118 | 6a | Mar. 8 | 112 |
| 5a | Dec. 30 | 182 | 4a | Mar. 24 | 48 |
| | | | 7a | Apr. 20 | 8 |
| | *1937* | | 4d | Apr. 27 | 3 |
| 5d | Jan. 8 | 20 | 4c | May 15 | 0 |
| 5b | Jan. 15 | 112 | 6c | May 15 | 0 |
| 4b | Jan. 22 | 116 | 6d | May 15 | 0 |
| 6b | Feb. 24 | 64 | | | |

[1] From both grass and soil.

## EXPERIMENT 3. LARVAE PLACED ON GRASS PLOTS AUGUST 5, 1937

The third experiment was initiated on August 5, 1937, by placing 50 gm. of crushed feces in the center of 12 subplots. Although the 50-gm. control cultures yielded an average of 10,640 larvae, the grass of a subplot examined on August 12, 8 days after the experiment was begun, yielded only 463 larvae. Soil and grass of a second subplot, examined on August 20, 1937, yielded 1,364 larvae. After the apparently rapid loss of larvae or eggs of *Haemonchus contortus*, as shown by the examination of August 12, it was believed that there were too few larvae left for the purposes of the experiment. Therefore, on August 20 additional larvae were placed on each remaining subplot in 70 gm. of feces that had been cultured for 4 days. An average of 27,265 larvae were recovered from 4 samples of 70 gm. each of the same lot of feces used on these plots. In this experiment the grass was not trimmed to keep it at a length of 4 inches. At intervals of about 1 week, soil and grass were removed from the subplots and examined, with the results shown in table 4. Except for collections made on October 2, the reduction in numbers of larvae recovered from the plots was constant and rapid. Larvae were found at all times not only on the surface of the soil, but also under the surface and on the grass.

TABLE 4.—*Number of Haemonchus contortus larvae recovered from grass and soil of subplots infected on August 5 and on August 20, 1937*

| Subplot No. | Date of collection | Larvae recovered from— | | | Total larvae recovered |
|---|---|---|---|---|---|
| | | Grass | Surface of soil | Soil to 1 inch deep | |
| | | *Number* | *Number* | *Number* | *Number* |
| 12a | Aug. 28 | 5,904 | 30 | 106 | 6,040 |
| 12b | Sept. 9 | 4,606 | 342 | 850 | 5,798 |
| 11d | Sept. 17 | 2,749 | 180 | ---------- | 2,929 |
| 11c | Sept. 23 | 1,402 | 63 | 167 | 1,632 |
| 11b | Oct. 2 | 3,944 | 576 | 1,920 | 6,440 |
| 11a | Oct. 9 | 784 | 50 | 24 | 968 |
| 7d | Oct. 17 | 122 | 21 | 100 | 243 |
| 7c | Oct. 30 | 18 | 1 | 5 | 24 |
| 7a | Nov. 6 | 22 | 4 | 29 | 55 |
| 7b | Nov. 18 | ---------- | 2 | 26 | 49 |

## EXPERIMENT 4. LARVAE PLACED ON GRASS PLOTS OCTOBER 11, 1937

In the first three experiments eggs and larvae were exposed to natural conditions, and the survivors were recovered from the plots as infective larvae. In the fourth experiment, to simplify the problem by eliminating consideration of survival of the eggs and preinfective stages, the larvae were kept in the laboratory until they had reached the infective stage and then were placed on the grass plots. For this study, infective larvae in 75 gm. of crushed feces were placed in the center of 16 subplots on October 11, 1937. Four lots of 75 gm. of feces each yielded an average of 38,500 larvae. Collections of material from the subplots were made at approximately weekly intervals. No samples of soil were taken from December to March while the ground was frozen. In the next to the last column of the table, the result shown for March 24 is for a pooled sample of the subplots indicated. The soil was removed in 1-inch layers to a depth of 3 inches, each inch of soil being examined for larvae separately. Four larvae were recovered from the 2-inch level and 5 from the 3-inch level. The number of larvae recovered from other samples is shown in table 5.

TABLE 5.—*Number of Haemonchus contortus larvae recovered from grass and soil of subplots receiving infective larvae on October 11, 1937*

| Subplot No. | Date of collection | Larvae recovered from— | | | | Total larvae |
|---|---|---|---|---|---|---|
| | | Grass | Feces | Soil surface | Soil 1 inch deep | |
| | *1937* | *Number* | *Number* | *Number* | *Number* | *Number* |
| 1a | Oct. 18 | 4,944 | 7,633 | 4,761 | 2,880 | 20,218 |
| 1b | Oct. 25 | 780 | 24 | 180 | 47 | 1,031 |
| 1c | Nov. 1 | 1,944 | 555 | 104 | 48 | 2,651 |
| 1d | Nov. 8 | 1,848 | 480 | 540 | 42 | 2,910 |
| 2a | Nov. 15 | 252 | 80 | 325 | 122 | 779 |
| 2b | Nov. 21 | 284 | 16 | 68 | 3 | 371 |
| 2c | Nov. 29 | 705 | 225 | 45 | 23 | 998 |
| 2d | Dec. 6 | 1,360 | 76 | 30 | [1] | 1,472 |
| | *1938* | | | | | |
| 3a | Jan. 15 | 140 | 83 | [1] | ---------- | 222 |
| 3b | Feb 6 | 112 | 26 | [1] | ---------- | 138 |
| 3c | Feb. 19 | 34 | 32 | [1] | ---------- | 66 |
| 3d | Mar. 5 | 35 | 4 | [1] | ---------- | 39 |
| 4a | Mar. 19 | 90 | 129 | [1] | ---------- | 319 |
| 4b | Mar. 24 | 22 | 9 | 31 | [2] 7 | [3] 62 |
| 4c | do | 37 | 8 | 29 | | [3] 64 |
| 4d | do | 24 | 9 | 31 | | [3] 64 |

[1] No samples taken as ground was frozen.
[2] Recovered from a pooled sample of soil from all 3 plots removed to a depth of 1 inch.
[3] Does not include soil sample 1 inch deep.

The reason for the low recovery of larvae in the latter part of October and the high recovery in the latter part of November and the first part of December is not known. The decrease in number of larvae recovered on the other dates indicates that death was constant and rapid. Here, too, as in experiment 3, larvae were always recovered from the grass, from the soil surface, and beneath the soil surface whenever the examination was made. There was an increase in number of larvae recovered on March 19, similar to the finding in experiment 1. In experiment 4, in which larvae were placed on the plots, 52.5 percent were recovered 7 days later; in experiment 1, in which eggs were used, only 3 percent were recovered as larvae 8 days later. The difference between 52.5 and 3 percent indicates a high death rate among eggs and preinfective larvae and a much lower death rate of infective larvae.

## SUMMARY AND CONCLUSIONS

Experiments were conducted from August 1936 to March 1938 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., to determine how long eggs and larvae of the large stomach worm (*Haemonchus contortus*) would survive under outdoor conditions during different seasons of the year. Feces of sheep infected with the parasite were placed on 2-foot-square grass plots in August and October.

During the fall and winter, infective larvae were recovered from the plots but in much smaller numbers than during the summer or early fall. In each of three tests continued until spring, the number of larvae recovered in March was higher than in the preceding month. This result probably was due to larvae being attracted to the surface of the soil by rising temperatures. A few infective larvae exposed on grass plots in August or October survived until the following spring, but all larvae surviving until April or May were sluggish and vacuolated and probably noninfective.

These findings indicate that pastures kept free of sheep from October until the middle of April will contain only a few larvae of *H. contortus* still capable of infecting these host animals.

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# FACTORS AFFECTING THE ASCORBIC ACID CONTENT OF CABBAGE LINES[1]

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## INTRODUCTION

Recent summaries of quantitative estimates of vitamin content of plant and animal foods give bewildering ranges in the readings. It has been shown that concentration of the same vitamin varies from tissue to tissue in the same organ (*3*),[2] from fruit to fruit on the same tree (*6*), from season to season on the same variety (*5*), and, of especial interest to the plant breeder, from variety to variety (*2*). A study of ascorbic acid content in cabbage from Ohio (*2*) showed that among 30 varieties grown there in the summers of 1938 and 1939 there were significant differences with average vitamin C content for 2 head samples each ranging from 0.48 mg. per gram of fresh weight for the variety All Head Select to 1.81 for Midseason Market, 2 varieties having practically the same average weight. This high reading of head leaves of the latter exceeds the previously recorded maximum of 1.58 mg. per gram for head leaves reported in 1939 from Texas (*4*); it places maximum readings for cabbage ahead of those for citrus juices, tomatoes, and strawberries as sources of ascorbic acid in the American dietary (*3*).

## MATERIALS AND METHODS

A study of some factors determining variability in ascorbic acid content in cabbage was conducted with 25 breeding lines planted in a randomized-block design having 3 replicates in the fall of 1941 and 3 in the spring of 1942. The randomized-block design permitted study of the variability of ascorbic acid readings with and without the correlated effect of head weight.

Variance of ascorbic acid alone was compared with the covariance of ascorbic acid on head weight; and covariance was found as the errors of estimate when the main terms were compared by Snedecor's method (*9, pp. 249–273*) with the error term. The mean unadjusted ascorbic acid readings were adjusted by the following formula:

$$X = Y - bx$$

where $Y$ = individual ascorbic acid readings
$b$ = regression coefficient of the error term
$x$ = departure of weight from the general mean
$X$ = adjusted individual ascorbic acid reading.

The breeding lines selected for this study are shown in table 1. All lines were obtained from survivors of the low temperatures of the fall of 1938.

[1] Received for publication February 27, 1943. This work was performed at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., chiefly under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] Italic numbers in parentheses refer to Literature Cited, p. 329.

Head characters were classified in the field at the time of harvest, and ascorbic acid content was obtained as soon as possible afterward by Morell's (*8*) rapid adaptation of the Bessey (*1*) and Mindlin and Butler (*7*) procedure. Samples were obtained by cutting the heads through the axis, removing an aliquot segment of each carefully with a large knife, and then macerating the tissue as described by Morell (*8*).

TABLE 1.—*Mean weight and ascorbic acid readings for 25 breeding lines of cabbage grown in 2 seasons, fall 1941 and spring 1942, together with adjusted ascorbic acid values*

| Breeding lines | Head weight | | Ascorbic acid per gram | | | | Ranking of adjusted values | |
|---|---|---|---|---|---|---|---|---|
| | | | Unadjusted | | Adjusted [1] | | | |
| | Fall | Spring | Fall | Spring | Fall | Spring | Fall | Spring |
| | *Pounds* | *Pounds* | *Milligram* | *Milligram* | *Milligram* | *Milligram* | | |
| Self-pollinated Volga: | | | | | | | | |
| 534-5-1-3 | 3.7 | 2.4 | 0.48 | 0.50 | 0.48 | 0.49 | 21 | 21 |
| 534-5-1-4 | 3.3 | 1.9 | .54 | .54 | .52 | .49 | 12 | 21 |
| 534-5-3-1 | 3.9 | 1.6 | .50 | .66 | .51 | .59 | 14 | 6 |
| 534-5-5-1 | 2.3 | 2.2 | .36 | .51 | .31* | .48 | 25 | 23 |
| 534-7-4-2 | 3.7 | 2.9 | .51 | .46 | .51 | .48 | 14 | 23 |
| 534-15-4-1 | 3.6 | 2.5 | .56 | .52 | .56 | .51 | 7 | 18 |
| 534-16-2-1 | 3.4 | 2.3 | .58 | .50 | .57 | .48 | 4 | 23 |
| Open-pollinated Charleston Wakefield ($F_3$): | | | | | | | | |
| 543-$0_1$-3-1 | 1.9 | 1.5 | .80 | .68 | .73* | .61 | 1 | 2 |
| 543-$0_1$-3-4 | 2.9 | 1.7 | .52 | .67 | .49* | .61 | 20 | 2 |
| 543-$0_1$-3-29 | 3.5 | 3.4 | .58 | .54 | .57 | .59 | 4 | 6 |
| 543-$0_1$-3-40 | 3.2 | 1.6 | .62 | .58 | .60 | .51 | 2 | 18 |
| 543-$0_1$-3-47 | 3.0 | 2.1 | .53 | .64 | .50* | .61 | 18 | 2 |
| 543-$0_1$-5-3 | 2.7 | 2.5 | .54 | .54 | .50 | .53 | 18 | 16 |
| 543-$0_1$-5-12 | 1.9 | 2.1 | .67 | .60 | .60 | .57 | 2 | 10 |
| 543-$0_1$-7-4 | 4.9 | 2.8 | .52 | .57 | .57 | .58 | 4 | 9 |
| 543-$0_1$-7-7 | 5.3 | 3.3 | .45 | .54 | .51 | .59 | 14 | 6 |
| 543-$0_1$-11-19 | 4.9 | 2.8 | .43 | .55 | .48 | .56 | 21 | 11 |
| 543-$0_1$-11-23 | 3.8 | 2.8 | .56 | .52 | .56 | .53 | 7 | 16 |
| 543-$0_1$-11-26 | 5.3 | 3.4 | .46 | .49 | .52 | .54 | 12 | 13 |
| 543-$0_1$-11-32 | 4.2 | 3.4 | .53 | .55 | .55 | .60 | 9 | 5 |
| Controlled cross (Volga×Charleston Wakefield): | | | | | | | | |
| BCC 2-1-10 ($F_3$) | 3.8 | 2.6 | .44 | .55 | .44* | .55 | 24 | 12 |
| Open-pollinated All Head Early: | | | | | | | | |
| 537-$0_1$-1-3 | 3.9 | 2.9 | .47 | .49 | .48 | .51 | 21 | 18 |
| All Head Select: | | | | | | | | |
| 539-$0_1$-1-1 | 5.1 | 2.4 | .48 | .55 | .53 | .54 | 11 | 13 |
| 539-$0_1$-5-3 | 3.5 | 2.7 | .52 | .53 | .51 | .54 | 14 | 13 |
| Wisconsin Ballhead: | | | | | | | | |
| 550-$0_1$-9-1 | 3.8 | 2.9 | .55 | .64 | .55* | .66 | 9 | 1 |
| General mean | 3.70 | 2.57 | .529 | .553 | .530 | .564 | | |

[1] * indicates that fall and spring values were significantly different. A difference of 0.11 mg. was necessary for significance at 5-percent point.

## RESULTS

Table 1 gives the mean head weights and the unadjusted and adjusted ascorbic acid readings for 25 breeding lines. The smaller average head weight of the spring crop, $2.57 \pm 0.07$ pounds, compared with that of the fall crop, $3.70 \pm 0.10$ pounds, was accompanied by a higher average ascorbic acid content, $0.553 \pm 0.007$ mg. In the fall the average content was $0.529 \pm 0.007$ mg. The differences between general means for the two variables are statistically significant at the 1-percent point, $1.13 \pm 0.12$ pounds for weight and $0.024 \pm 0.009$ mg. for ascorbic acid.

Table 2 shows $F$ values for the variance in ascorbic acid and for the covariance of ascorbic acid on head weight for the two seasons singly

and combined. The standard error of a single determination, 0.067 in the fall and 0.062 in the spring, and the correlations between weight of head and ascorbic acid, viz −0.55**[3] in fall and −0.61** in spring for error sources of variance, are practically the same. Variance for lines is highly significant, but that for replicates is not significant.

When seasons are combined, there is a highly significant seasonal effect in the variance of ascorbic acid, 7.88**, but when regression on weight is considered as in the covariance of season compared with error, the significance is eliminated, 2.97. The significance of the variety term in variance, 5.90**, and covariance, 5.51**, is above the 1-percent point. Ascorbic acid readings are adjusted to the regression data for general mean head weight in columns 6 and 7 of table 1.

TABLE 2.—*Analysis of variance and regression data for values in table 1*

| Sources of variability | Variance | | Regression data | | | |
|---|---|---|---|---|---|---|
| | Degrees of freedom | *F* values [1] | Degrees of freedom | *F* values for covariance [2] | Regression coefficient | Correlation coefficient |
| Fall 1941: | | | | | | |
| Lines | 24 | 5.04** | | 5.22** | | |
| Replicates | 2 | .84 | | .22 | | |
| Error | | | 47 | | −0.0378 | −0.5527** |
| Total | | | 73 | | −.0434 | −.5282** |
| Spring 1942: | | | | | | |
| Lines | 24 | 2.85** | | 2.79** | | |
| Replicates | 2 | 2.15 | | 1.25 | | |
| Error | | | 47 | | −.0669 | −.6144** |
| Total | | | 73 | | −.0695 | −.6348** |
| Both seasons: | | | | | | |
| Lines | 24 | 5.90** | | 5.51** | | |
| Replicates [3] | 4 | 1.45 | | .97 | | |
| Season | 1 | 7.88** | | 2.97 | | |
| Seasons × lines [2] | 24 | 2.14** | | 2.59** | | |
| Error | | | 95 | | −.0453 | −.5607** |
| Total | | | 148 | | −.0448 | −.5597** |

[1] Standard errors for a single determination are fall, 0.067; spring, 0.062; both, 0.065.
[2] *F* values for covariance when the interaction term is used instead of the error term are for lines 2.06*, and for season, 0.53.
[3] Variability of replicates within seasons.
**=Significant at the 1-percent point.

Inconsistency in behavior of these 25 lines in the 2 seasons is shown by the *F* value of 2.06* for the comparison (footnote 2, table 2) of the covariance for lines with the interaction term. The details of the inconsistency are seen in the columns of table 1 giving mean unadjusted and adjusted ascorbic acid values. Furthermore, the coefficient of correlation between 1941 and 1942 unadjusted mean readings is 0.4616*, whereas between the adjusted readings it is 0.3353.

## DISCUSSION

Table 2 shows a highly significant degree of negative correlation between ascorbic acid and head weight, −.5607**, for the combined-seasons error term; this is low enough, however, to indicate that head weight is not a major factor in affecting concentration of ascorbic acid.

The relative importance of heredity (line sources of variability) and environment (replicates and seasons) in producing ascorbic acid

* Values marked ** are statistically significant at the 1-percent point; those marked * at the 5-percent point.

differences between breeding lines is shown in table 2 by the relative sizes of $F$ values for variance and covariance analyses. Column 3 shows these relative values when the effect of head weight is not eliminated (variance), and column 5 when it is (covariance).

The $F$ values for variance suggest that seasonal effect, 7.88**, is more important than line effect, 5.90**, and that both effects are significant at the 1-percent point, whereas no significance is attached to replicates (soil variability). The $F$ values for covariance, where head weight influence is eliminated, show highly significant line variability, but nonsignificant seasonal variability. In other words, variability in ascorbic acid between breeding lines is determined mainly by hereditary factors, and seasonal influence operates mainly on head size and therefore to a lesser degree on ascorbic acid content.

Comparison of the covariance for lines with that of the interaction term discloses a significant degree of inconsistent varietal (line) behavior from season to season. The extent of inconsistency is shown after adjustment of the ascorbic acid readings according to the regression coefficients for the two seasons in the last four columns of table 1. The new values in ascorbic acid content are best shown by their relative rankings.

There is greater variability in ascorbic acid in the fall than in the spring, and some lines apparently do better in one season than another. Application of the size of a significant difference between seasons, 0.11 mg., to the data for adjusted mean readings in ascorbic acid discloses six significant differences. Most, but not all, of these differences favor the spring. The outstanding exception is line 543–$0_1$–3–1, which, although always near the lead, apparently does significantly better in the fall.

As far as temperature is related to ascorbic acid there appears to be evidence that the ultimate effect, whether enhancing or depressing, depends on the genetic constitution of the line in question.

A noteworthy line is 550–$0_1$–9–1, which in addition to being consistently high in ascorbic acid, is also above average in head weight and serves, with lines 543–$0_1$–7–4 and 543–$0_1$–11–32, to demonstrate that small weight is not a primary factor in producing high ascorbic acid.

## SUMMARY

There is a significant degree of negative correlation between ascorbic acid concentration and head weight among 25 breeding lines of cabbage grown as 3 replicates in the fall and 3 in the spring.

Of the six lines with more consistently high ascorbic acid content, judged by adjusted ranking, three were above average in head weight.

Regression data show that hereditary differences are more important than seasonal or positional differences in producing ascorbic acid differences between breeding lines.

Seasonal effect on ascorbic acid is expressed chiefly on head size and secondarily on genetic constitution.

Only six lines differed significantly in ascorbic acid content from season to season. In only one of these was the content higher in the fall than in the spring.

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